Persistence of residual diphacinone concentrations in pig tissues following sublethal exposure

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ABSTRACT

Effective but less persistent alternatives to brodifacoum are being sought for the control of introduced rats (*Rattus* spp.) on mainland New Zealand. The first-generation anticoagulant diphacinone is currently being investigated in this context, including assessment of the potential risks of environmental contamination and secondary poisoning. A pen trial was conducted to address information gaps regarding the toxicity and residual persistence of diphacinone in pigs (*Sus scrofa*). Significant elevations in prothrombin time, International Normalised Ratio values and activated partial thromboplastin time were measured in pigs at day 2 following oral diphacinone doses of 12.5 mg/kg, 0.25 mg/kg/day for 3 days, or 0.5 mg/kg/day for 5 days. These values had returned to pre-dosing levels by day 7 in all pigs except two, which were euthanased during the trial due to increasing lameness; at necropsy, these two pigs were found to have severe haemorrhage in or around a leg joint. After exposure of pigs to a sublethal diphacinone dose (12.5 mg/kg) in food, elimination half-lives of residual diphacinone in liver, muscle and fat were 5.43–14.12 days, 4.48 days and 2.29 days respectively. These figures suggest that c. 160 days would be a conservative withholding period before feral pigs are taken for human consumption in areas where diphacinone baits have been used, to minimise the likelihood of detectable diphacinone residues (≥ 0.02 µg/g) occurring in wild pork.

Keywords: diphacinone, pigs, *Sus scrofa*, anticoagulant, coagulation time, residues, liver, meat, half-life

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1. Introduction

Effective, less persistent alternatives to the vertebrate pesticide brodifacoum are being sought for the control of field populations of rats (*Rattus* spp.) on mainland New Zealand. The first-generation anticoagulant diphacinone has been shown to be less persistent in rat liver (Fisher et al. 2003), and has a lower acute toxicity to mammals and birds than brodifacoum (EPA 1998). Diphacinone is currently being registered in the United States for broad-scale field application in rodent control (e.g. Johnston et al. 2005) and has been undergoing assessments of field efficacy against rats in New Zealand (Gillies 2006). Besides assessing cost-efficacy of diphacinone bait formulations for broad-scale rodent control in New Zealand, there are information gaps that need to be addressed before an adequate assessment of the likely risks of diphacinone use can be made. These include the potential effects of diphacinone on non-target wildlife species that may be exposed to it, and the environmental persistence and fate of residual diphacinone.

Feral pigs (*Sus scrofa*) in New Zealand sometimes access toxic baits laid for possum (*Trichosurus vulpecula*) or rodent (rat and mouse *Mus musculus*) control by destroying bait stations (primary exposure), and may also consume contaminated carcasses of poisoned target animals (secondary exposure) (Morriss et al. 2005). In the instance of a relatively high but sublethal diphacinone intake by a pig, the residual concentration and persistence of diphacinone that may occur in tissues is unknown. This study aimed to address this information gap by investigating the toxicity and persistence of residues of diphacinone in captive pigs. Evaluating the persistence of diphacinone in pigs, after simulating the intake that could be expected as a result of accessing bait stations, will allow a withholding period for harvesting feral pigs for meat to be defined, thus supporting the risk assessment processes used by the Department of Conservation (DOC) in applying diphacinone for field rodent control.

Studies of diphacinone in pigs include an assessment of acute oral (single-dose) toxicity, which produced an LD_{50} estimate of > 150 mg/kg (Hazelton Laboratories Inc. 1957). This estimate was made on the basis of three pigs dosed with 27 mg/kg, 150 mg/kg and 0 mg/kg respectively. However, since both pigs that received diphacinone survived, statistical confidence intervals could not be calculated. This single-dose toxicity value for pigs is relatively high compared with single-dose LD_{50} estimates for diphacinone for other mammals, such as dogs (*Canis lupus familiaris*: 3–7.5 mg/kg; Mount & Feldman 1983) and Norway rats (*Rattus norvegicus*: 1.93–43.3 mg/kg; Jackson & Ashton 1992). It is unclear whether pigs have a particularly low single-dose susceptibility to diphacinone, or whether the available toxicity estimate of > 150 mg/kg needs to be refined.

In a recent study in the United States, Fletcher (2002) found that pigs fed with diphacinone at 0.133 mg/kg/day for 7 days all survived without any obvious signs of poisoning. However, pigs fed with 0.333 mg/kg/day for 7 days showed some signs of poisoning and haemorrhage pathology on necropsy 21 days later. In an earlier study, Keith et al. (1990) offered pigs diphacinone in food at doses of 0.6 mg/pig/day for 2 days and 1.5 mg/pig/day for 5 days. These pigs showed no signs of poisoning and had normal coagulation times 2 days and 10 days after dosing. However, since no body weights were given for the pigs, the exact concentrations (mg/kg) of diphacinone they received are unknown. Given that a diphacinone dose of 0.333 mg/kg/day produced obvious signs of poisoning, followed by recovery (Fletcher 2002), the multiple-dose LD_{50} for pigs is likely to be higher than this. However, even the single acute toxicity estimate of > 150 mg/kg is very general and does not allow a precise estimation of what might constitute lethal and sublethal intakes of diphacinone in pigs. These previous studies have focused on the toxicity of diphacinone to pigs, and the residue data reported in them do not allow adequate assessment of the persistence of diphacinone residues in pig tissue.

In this study, a pilot evaluation was undertaken with domestic pigs to gauge sublethal toxicity, measured as a response in coagulation times, of single and multiple oral doses of diphacinone. The toxic action of anticoagulants is largely through the inhibition of Vitamin-K-dependent blood-clotting factors, with resultant prolonged coagulation times to the point where lethal haemorrhage occurs. The prothrombin time (PT) blood test is commonly used to monitor oral anticoagulant use in human medicine and is sensitive to Blood Factors II, VII and X (part of the extrinsic clotting pathway) (e.g. Poller & Hirsh 1996). The activated partial thromboplastin time (APTT) is a screening test to detect abnormalities in the intrinsic coagulation system (Factors II, V, VII, IX, X, XI and XII) (e.g. Karges et al. 1994). Together, these tests can indicate the degree to which coagulation time has been affected by a dose of anticoagulant, with extremely prolonged coagulation times indicating a toxic effect.

The main objectives of this study were to estimate the sublethal toxicity of diphacinone to pigs, and then to measure the persistence of residual concentrations of diphacinone in pig tissues following sublethal oral exposure. Testing for a dose-response in coagulation times of pigs given different doses of diphacinone indicated what exposure constitutes a high sublethal dose. Establishment of this dose value was then used as a starting point from which to measure the elimination rate of residual diphacinone in liver, and also in those pig tissues most likely to be used for human consumption, i.e. muscle and fat.

2. Methods

2.1 PIG HOUSING AND HUSBANDRY

Young domestic weaner pigs (c. 8 weeks old; equal sex ratio), individually identified with numbered plastic eartags, were obtained from a commercial piggery. They were housed in groups of 12 in a pen (10 m × 12 m) in a large shed, with wood shavings over a concrete floor and a roofed sleeping area constructed of hay bales. Water was freely available through an automatic drinker, and pigs were fed a twice-daily individual ration of commercial pig feed (Weston Animal Nutrition, Rangiora) to approximate a daily intake of 5% of their body weight. Weaner pellets were fed until 13 weeks of age, and thereafter commercial grower & finisher pellets. The weaner feed contained Vitamin K_3 at 2.5 g/tonne, and the grower & finisher feed at 2 g/tonne. This was not expected to compromise the effect of diphacinone on pigs, as Vitamin K_1 is the antidotal form for anticoagulant poisoning and was not present in the food. Pigs were regularly weighed using trailer-mounted stock scales to monitor their growth and general health.

Twelve individual feeding bays formed one wall of the pen. For at least 1 week before the trial, pigs were acclimatised to entering a bay and being shut in at feed times. Alongside their morning ration of pellets, pigs were also offered another palatable food (a c. 30-g ball of sugar dough), to accustom them to the vehicle used to deliver the allocated dose of diphacinone during the trial.

2.2 ESTIMATION OF HIGH SUBLETHAL INTAKES OF DIPHACINONE IN PIGS

2.2.1 Dosing and blood sampling

Baseline blood samples were taken 1 week before dosing with diphacinone from 11 of the 12 pigs in the trial; one pig was not sampled because it had been treated for an infection 2 days beforehand. For sampling, pigs were placed on their backs under restraint, and up to 10 mL of blood was drawn from the anterior vena cava of each pig, using a 10-mL syringe and 18G × 1 ½" needle. Samples were divided into two 4.5-mL blood collection tubes containing 0.129 mol/L tri-sodium citrate solution (Vacutainer® Blood Collection Tubes, 9NC, 3.8% sodium citrate, Becton Dickinson) and stored on ice until centrifugation to obtain plasma for coagulation time testing.

Pigs were randomly allocated to treatment groups and weighed to determine individual doses of diphacinone that were approximations of possible field exposures. For example, the largest bait stations used in field application of rodenticides generally hold 2 kg of 50-ppm diphacinone bait. Therefore, if a 40-kg pig were to access the contents of such a station, it would receive a dose of 2.5 mg/kg diphacinone. Doses were prepared by uniformly mixing the appropriate amount of diphacinone powder (Animal Control Products, 1.98% diphacinone as assayed by the Landcare Research toxicology laboratory) into c. 30 g of the vehicle (sugar dough) and making it into a ball. Doses were offered to pigs

alongside their morning feed on appropriate day(s) (Table 1) and consumption was recorded. Pigs were returned to their normal diet after the allocated dose was eaten, or if it was refused for more than 1 h. They were then observed for the following 3 weeks for any signs of poisoning, e.g. haemorrhage, anemia or inappetance. Blood samples were taken as described above, at days 2, 7 and 14 after dosing, for coagulation-time testing.

Contingency planning to avoid death by toxicosis as an experimental endpoint included immediate (within 6 h) testing of the day 2 and day 7 blood samples for elevated blood coagulation times, combined with close (at least twice daily) observation of pigs during the 7 days immediately after dosing. In the event that a pig showed signs of anticoagulant poisoning and had a significantly elevated coagulation time, a licensed captive-bolt gun operator was available to carry out euthanasia.

TABLE 1. DIPHACINONE DOSING SCHEDULE FOR DOMESTIC PIGS (*Sus scrofa*) IN THE INITIAL TRIAL TO ESTIMATE HIGH SUBLETHAL DOSES. F = FEMALE; M = MALE.

NO. PIGS (SEX)	DIPHACINONE DOSE (NO. APPLICATIONS)		EXPOSURE SCENARIO
3 (2F, 1M)	12.5 mg/kg	(single)	Pig accesses the contents of five bait stations within 1 day
3 (1F, 2M)	2.5 mg/kg	(single)	Pig accesses the contents of one bait station in 1 day
3 (1F, 2M)	2.5 mg/kg/day	(3 days)	Pig accesses the contents of one bait station per day for 3 days
3 (2F, 1M)	0.5 mg/kg/day	(5 days)	Pig accesses c. 400 g of bait per day for 5 days

2.2.2 Measuring blood coagulation times

Within 2 h of sampling, both whole-blood aliquots from each pig were centrifuged at 2500*g* for 15 min at 4°C. Plasma samples were aliquoted into Eppendorf tubes and stored at –80°C if testing could not be carried out that day, but more usually at –4°C when testing could be completed within 4 h. Where thawing was required before testing, samples were placed in a hot water bath at 37°C. One plasma aliquot from each pig at each sample interval was retained at –80°C, in case additional testing was required.

Duplicates from plasma samples were tested using commercial PT (Simplastin® Excel S, BioMèrieux Inc., USA) and APTT (Platelin®, BioMèrieux Inc., USA) testing kits and an automated coagulometer (Amelung KC4Amicro, Sigma Diagnostics). The commercial kits included control plasma reagents, which provided internal validation standards for the pig plasma samples. The PT times were converted to International Normalised Ratio (INR) values, which were calculated as the ratio of the mean PT value obtained from duplicate testing of the plasma from a diphacinone-treated pig, to the mean baseline PT (calculated from all pigs from which pretreatment blood samples were obtained), then raised to the power of the International Sensitivity Index (ISI) figure. The ISI is used as a correction factor to account for the response of different thromboplastins to oral anticoagulants, and in this instance was 1.23 for the Simplastin® Excel S kit (BioMèrieux Inc., USA).

2.3 PERSISTENCE OF RESIDUAL CONCENTRATIONS OF DIPHACINONE IN PIG TISSUES

Twelve weaner pigs (six male, six female, Hamdam cross breed) were group-housed and acclimatised, as described in section 2.1, until they were administered a single dose of 12.5 mg/kg diphacinone in a dough ball. This dose represented a high sublethal exposure, based on coagulation-time responses to different diphacinone doses measured in pigs in the previous trial (see section 3). Pigs were monitored at least once daily for signs of anticoagulant toxicosis until tissue sampling was completed. Pigs were randomly allocated to three groups with equal sex ratios ($n = 4$), and were sampled on days 1, 4 or 10 after dosing.

Tissue sampling was carried out immediately after pigs were euthanased by a licenced operator using a captive-bolt gun. Pigs were bled by severing the carotid artery and jugular vein, and whole-blood samples (at least 10 mL) were collected from this flow into citrate tubes. Livers were removed from the carcasses and weighed, and subsamples (c. 50 g) of each tissue type were retained alongside samples of muscle (from the rear haunch) and abdominal fat. Tissue samples were stored at –20°C until residue analysis could take place. Blood samples were prepared and stored frozen as described previously.

2.3.1 Analysis of tissue samples for diphacinone concentration

Concentrations of diphacinone in pig tissues (liver, muscle and fat) were determined by the Landcare Research toxicology laboratory, Lincoln. A sample of tissue (1 g) was weighed into a glass tube. Chlorophacinone (100 µL; as an internal standard) and anhydrous sodium sulphate (5 g) were added, followed by chloroform/acetone/formic acid (0.25%, 35 mL), and the contents of the tube were shaken and centrifuged. The supernatant was decanted and the extraction process repeated twice more. The combined extracts were evaporated and taken up in hexane/chloroform for clean-up on a carbograph SPE column, followed by an aminopropyl column. The analyte was eluted from the aminopropyl column with mobile phase A, which was then evaporated to dryness and taken up in mobile phase for quantification by HPLC analysis, using a C8 10-µm column and UV detector set at 284 nm. A method detection limit (MDL) of 0.02 µg/g and analysis uncertainty (95% confidence interval) of ± 20% was estimated according to TLM067 (IANZ-registered toxicology laboratory, Lincoln).

2.3.2 Statistical analysis

The blood-coagulation-time data were analysed using procedure LME in the statistical package R (R Development Core Team 2004). Linear mixed models were fitted to test for significant differences in the response variables (PT, PT-INR and APTT) with respect to diphacinone dose group (12.5 mg/kg, 2.5 mg/kg, 2.5 mg/kg/day for 3 days, or 0.5 mg/kg/day for 5 days) and days after dosing (14, 7, 2 or 0/baseline). Pigs were treated as random effects, while fixed effects were dose group and days after dosing. An autocorrelation function was fitted to the data, as repeated blood samples were taken from the same pigs; however, this was not needed in the model and was subsequently removed. The data from the second pig euthanased (on day 6 after dosing) were omitted from the analysis because its death did not fall into one of the 'days after dosing' classifications. Statistical analyses of the tissue residue data from the second

trial were carried out using GenStat Committee (2002). An exponential decay model was used to derive equations and estimate half-life figures for residual concentrations of diphacinone in liver, muscle and fat from pigs that had received a 12.5-mg/kg dose. For the purposes of making conservative estimates of the persistence of diphacinone, tissue samples in which the measured concentration was below the MDL were included in the data as having 0.02 μg/g residual diphacinone.

3. Results

3.1 COAGULATION TIME RESPONSES FOLLOWING INTAKES OF DIPHACINONE IN PIGS

The 12 pigs (six male, six female) in this trial gained weight steadily during the acclimatisation and trial period (Fig. 1). The use of an accustomed palatable food (dough balls) as a vehicle to dose pigs with diphacinone was generally successful, with all pigs except one eating the entire dose within 15 min of it being offered. One pig was slower than others to consume dough throughout the acclimation period, and this was partly overcome by mixing the uneaten portions with a small amount of normal pellet feed, which resulted in at least 80% of the intended dose (0.5 mg/kg/day for 5 days) being consumed within an hour on each day of dosing. The other two pigs in the 0.5 mg/kg/day for 5 days treatment group were euthanased during the trial due to increasingly severe lameness. The first of these, a female, was euthanased on day 2 and the second, a male, was euthanased 3 days after this (day 6). Necropsy of both euthanased pigs revealed a severe haemorrhage spreading from the main joint of the affected leg upwards to form an extensive haematoma along the bone, which presented as visible swelling. Elevated PT (64.55 s and 311.25 s) and APTT (88.3 s and 149.85 s) were measured in blood samples taken immediately post-mortem from the two

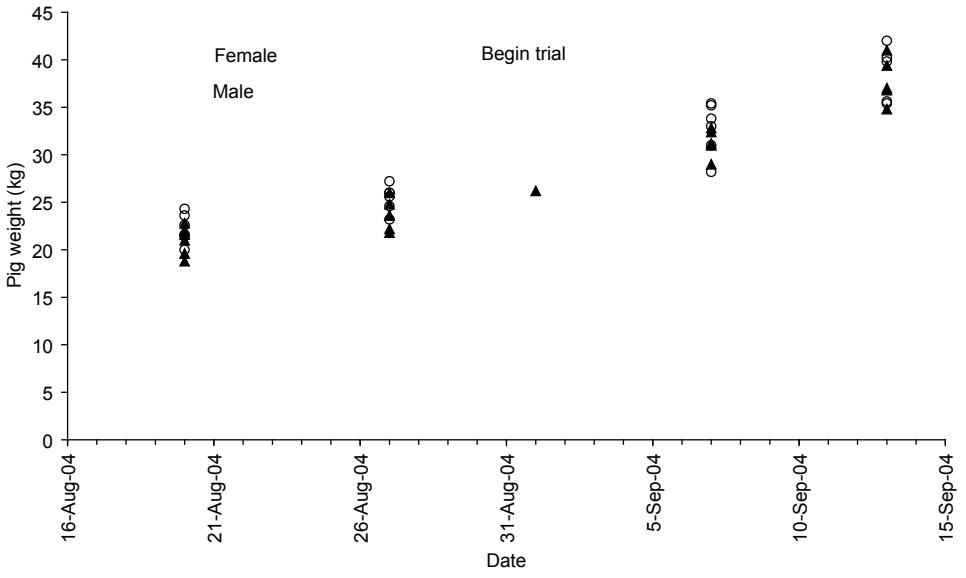


Figure 1. Weight gain in six male and six female weaner pigs (*Sus scrofa*) brought into group-housing, and dosed with diphacinone as per Table 1. The single data point at 01-Sep-04 is the first pig that was euthanased during the trial.

euthanased pigs, with INR values of approximately 6 and 49 respectively. Liver diphacinone concentrations were 0.7 µg/g and 0.12 µg/g, respectively, but were not included in further analyses.

The mean baseline PT value was 14.94 s, and the mean baseline APTT value was 33.35 s. Individual baseline INR values ranged from 0.89 to 1.14, as would be expected when using the mean baseline PT derived from all pigs in the trial to calculate INR. Initially, day after dosing and dose were included as fixed factors in the model along with their interaction. However, neither the interaction nor the effect of dose group was significant in any of the analyses (all $P > 0.05$), so these were not included in subsequent models. All of the diphacinone doses administered to pigs caused a significant elevation in coagulation time, but there was no evidence of a dose-related response. In the model, only day had a significant effect on PT, INR and APTT: for each of these variables, values at day 2 were significantly higher than baseline; subsequent values at days 7 and 14 were not significantly different from baseline (Figs 2–4).

Necropsy of the other ten pigs surviving at the completion of this trial (2 weeks after dosing commenced) did not show any gross evidence of severe haemorrhage of internal organs or limbs. In two pigs, however, healing subcutaneous haemorrhages were present on the inner rear legs, which were thought to have occurred during earlier restraint for blood sampling, possibly while the pigs were affected by anticoagulant dosing. There was no significant difference in the liver concentrations of residual diphacinone at 15 days between the dose groups ($F = 4.25$, df = 3, 6, $P = 0.06$), and the mean residual diphacinone concentration in livers was 0.44 µg/g, providing a preliminary indication that the liver persistence of diphacinone was at least 2 weeks.

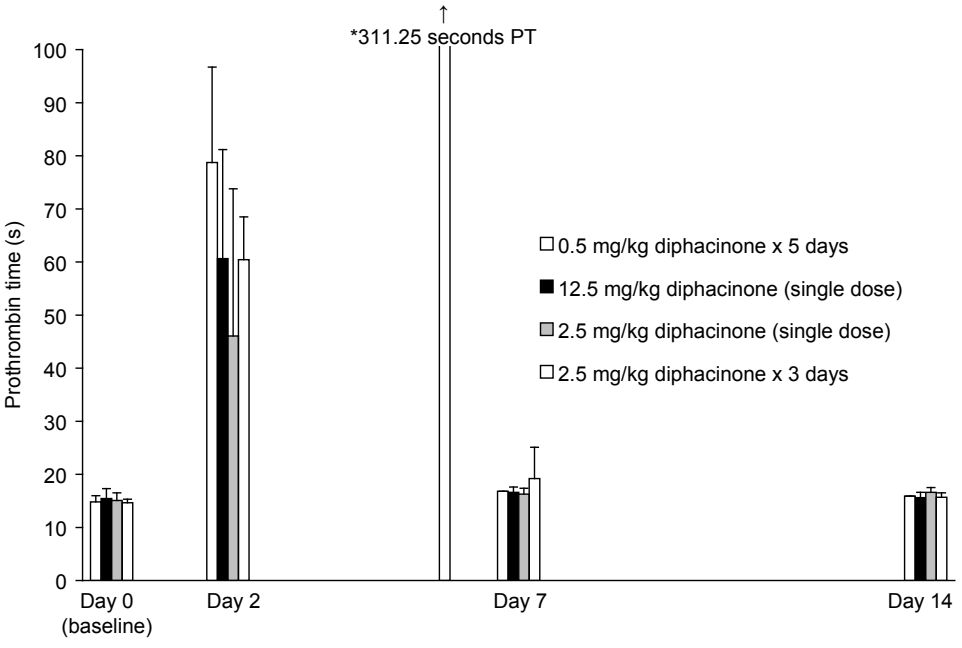


Figure 2. Mean prothrombin times (and upper 95% confidence intervals) in pigs dosed with either 12.5 mg/kg, 2.5 mg/kg, 2.5 mg/kg/day for 3 days, or 0.5 mg/kg/day for 5 days. Values on day 2 were significantly different from those on days 0, 7 and 14 ($F = 62.3$, df = 3, 27, $P < 0.001$). Includes data from the one pig euthanased at day 2 due to lameness (in the 0.5 mg/kg × 5 days group), and shows a very high PT value for a second pig euthanased on day 6 (*).

Figure 3. Mean International Normalised Ratio (INR) values (upper 95% confidence intervals), derived from prothrombin times in pigs dosed with either 12.5 mg/kg, 2.5 mg/kg, 2.5 mg/kg/day for 3 days, or 0.5 mg/kg/day for 5 days. Values on day 2 were significantly different from those on days 0, 7 and 14 ($F = 51.1$, df = 3, 27, $P < 0.001$). Includes data from the one pig euthanased at day 2 due to lameness, but excludes data from a second pig euthanased on day 6.

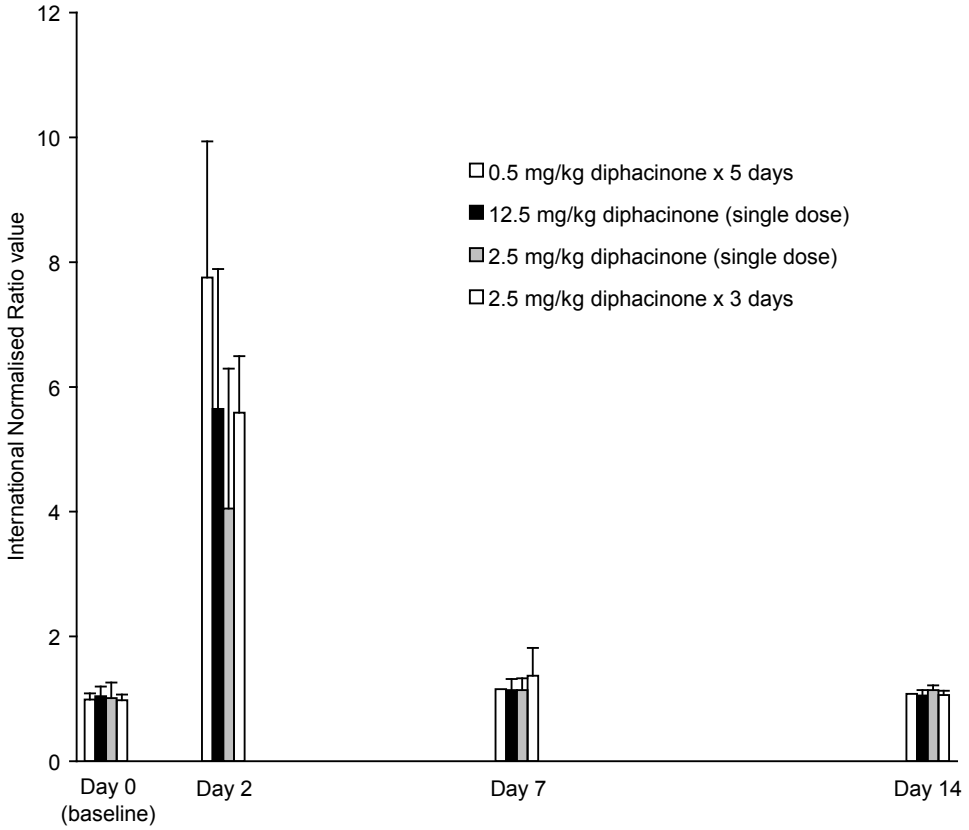
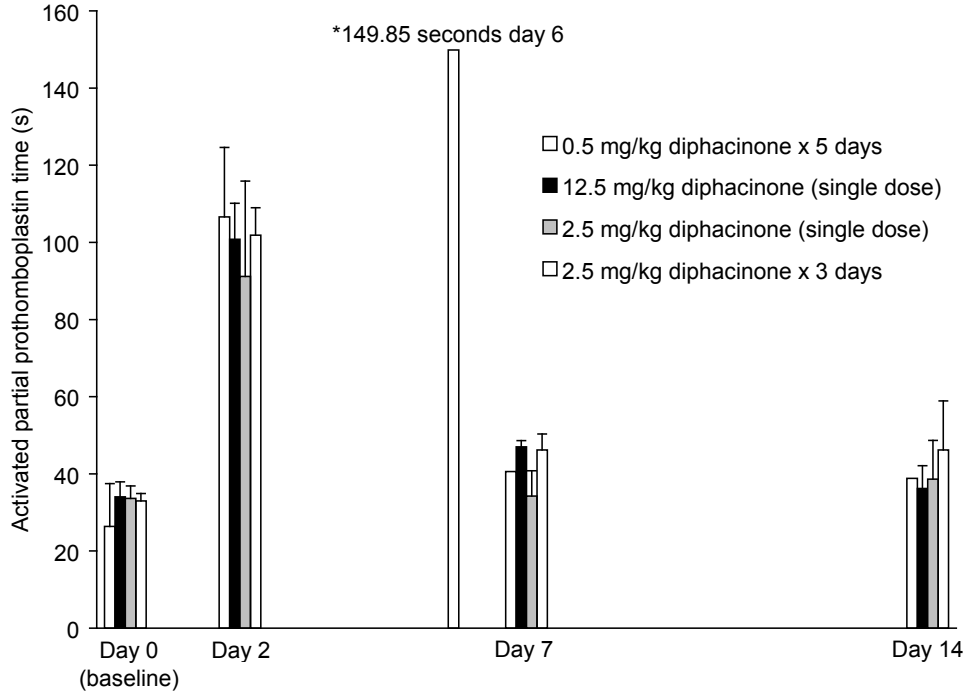


Figure 4. Mean activated partial thromboplastin times (APTT; upper 95% confidence intervals), in pigs dosed with either 12.5 mg/kg, 2.5 mg/kg, 2.5 mg/kg/day for 3 days, or 0.5 mg/kg/day for 5 days. Values on day 2 were significantly different from those on days 0, 7 and 14 ($F = 134.6$, df = 3, 26, $P < 0.001$). Includes data from the one pig euthanased at day 2 due to lameness, and data from a second pig euthanased on day 6 (*).



3.2 PERSISTENCE OF RESIDUAL DIPHACINONE IN PIG TISSUES

Table 2 shows the diphacinone residues detected over time in pig liver, muscle and fat following an oral dose of 12.5 mg/kg; these data were used to calculate half-life estimates. Samples taken at 'day 15' are from the three pigs dosed with 12.5 mg/kg diphacinone in the preceding sublethal dose trial. Figure 5 shows the half-life curves for diphacinone in each tissue. In liver, the elimination half-life of diphacinone was 5.43 days (95% CI: 3.55–11.52 days); in muscle, the half-life was 4.48 days (95% CI: 3.16–7.68 days); and in fat, the half-life was 2.29 days (95% CI: 1.66–3.68 days). Since the elimination of anticoagulants from the liver typically undergoes a rapid initial phase followed by a less steep terminal phase (Parmar et al. 1987), separate half-lives for diphacinone in liver were calculated for residue concentrations measured at days 1–4 (1.30 days; 95% CI: 0.84–2.88 days), and for those at days 4–15 (14.12 days; 95% CI: 5.34–? days—upper 95% confidence limit not defined).

TABLE 2. DIPHACINONE RESIDUES DETECTED OVER TIME IN PIG (*Sus scrofa*) LIVER, MUSCLE AND FAT FOLLOWING A SINGLE ORAL DOSE OF 12.5 mg/kg. F = FEMALE; M = MALE.

PIG ID/SEX	DAY	DIPHACINONE RESIDUE (µg/g)		
	POST DOSING	LIVER	MUSCLE	FAT
400/F	1	2.45	0.37	0.38
395/M	1	2.81	0.22	0.43
398/F	1	2.82	0.29	0.44
393/M	1	3.22	0.36	0.6
392/M	4	0.45	0.1	0.088
397/F	4	1.46	0.043	0.042
396/M	4	0.27	0.17	0.046
351/F	4	0.59	0.13	0.037
352/F	10	0.59	0.2	<MDL*
394/M	10	0.53	0.081	<MDL*
339/F	10	0.61	0.12	0.028
391/M	10	0.75	0.1	0.025
28/F	15†	0.18	<MDL‡	–
43/M	15†	0.41	<MDL‡	–
25/F	15†	0.4	<MDL‡	–

* Method Detection Limit for diphacinone in fat = 0.02 µg/g.

† From the first (sublethal dose) trial.

‡ Method Detection Limit for diphacinone in muscle = 0.02 µg/g.

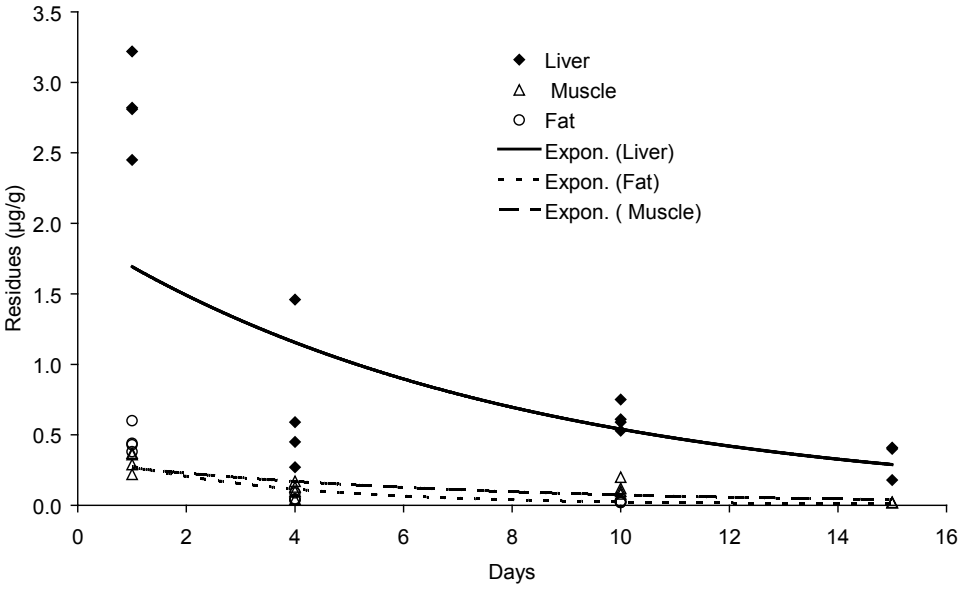


Figure 5. Residual diphacinone concentrations in pig (*Sus scrofa*) liver, muscle and fat following an oral dose of 12.5 mg/kg. Exponential decay equations are: liver $y = 1.923e^{-0.1276x}$; muscle $y = 0.2999e^{-0.1547x}$; fat $y = 0.3669e^{-0.3029x}$. Note this figure incorporates data from three pigs dosed with 12.5 mg/kg and euthanased at day 15 in the first trial.

4. Discussion

The baseline values obtained for PT in pigs (mean 14.94 s) in this study were generally comparable to mean PT obtained for pig plasma in studies using different test systems: 13.2 s (Hahn et al. 1996), 11.4 s and 12.8 s (McGlasson et al. 1998), and a median of 18.6 s (Munster et al. 2002). The mean baseline APTT value of 33.35 s measured in this study was consistent with a previously reported value for mean normal APTT in pigs (34.5 s; Drescher et al. 2002). However, other researchers have reported 15.2 s for mean APTT in anaesthestised pigs (Hahn et al. 1996), 15.8 s for mean baseline APTT in conscious miniature swine and 23.8 s for median APTT in blood from unanaesthetised pigs (Munster et al. 2002). The latter authors investigated the applicability of commercially available human coagulation assays for use with porcine plasma, and concluded that they could be validly used. However, in terms of measuring a response to the diphacinone doses in this trial, it was more meaningful to examine INR values. In human medicine, a 'safe' therapeutic range of INR values is generally quoted as 2–3; INR values greater than 5 indicate an anticoagulation response where haemorrhage could possibly occur. On this basis, the diphacinone doses ingested by pigs in this study all caused a potentially lethal degree of anticoagulation at day 2, which returned to 'normal' values in most cases by day 7 (Fig. 3). McGlasson et al. (1998) dosed pigs daily for 14 days with 2–3 mg of coumadin (equivalent to c. 0.07–0.08 mg/kg/day of warfarin) and measured PT and APTT values at days 0, 7 and 14, using two different test systems. Pigs responded to the coumadin doses with increased APTT times at 7 days (28.0 s) and 14 days (53.0 s) and INR values above 5 at 14 days (the actual value depending on the test system used), although McGlasson et al. (1998) did not document any visible signs of anticoagulation.

Lameness is a commonly reported early sign of anticoagulant toxicosis in pigs (e.g. Dobson 1973; Hone & Kleba 1984; O'Brien & Lukins 1990). Factors such as individual movement and the presence of existing injury probably influences

whether a severe haemorrhage occurs once coagulation times are significantly elevated above normal. An increased duration of elevated coagulation times is also likely to be important in determining the likelihood of severe haemorrhage. The results of this pen trial indicate that pigs are more susceptible to diphacinone than suggested by existing toxicity data, and that the acute toxicity of diphacinone to pigs is increased when it is ingested as multiple consecutive doses over time, as is typical of the first-generation anticoagulants. The primary poisoning risks to feral pigs in New Zealand resulting from other single and multiple exposures to diphacinone baits in the field remain to be evaluated. However, feral pigs are capable of consuming sufficient diphacinone bait to cause mortality if access is allowed, as evidenced by recent data from field trials of diphacinone in Hawai'i showing significant mortality of radio-collared pigs and tissue residues in surviving pigs (Pitt et al. 2004).

This study reports the highest residual concentrations of diphacinone measured in pig liver and muscle (3.22 μg/g and 0.37 μg/g respectively) to date, and these occurred in the same pig at 1 day after dosing with 12.5 mg/kg. In a study in Hawai'i of feral pigs that were exposed to a trial field application of diphacinone bait, one pig also had the highest diphacinone concentrations detected for both liver and muscle (3.07 ppm (equivalent to μg/g) and 0.12 ppm respectively; Pitt et al. 2004). In contrast, the highest residual diphacinone concentration in pig liver reported by Keith et al. (1990) was 0.83 ppm.

Using the half-lives estimated for diphacinone in muscle and fat, it would take 19 days and 10.5 days respectively for the highest residue measured in these tissues (0.37 μg/g and 0.43 μg/g) to decline below detectable concentrations. Using the terminal half-life estimate of 14.12 days for diphacinone in liver, it would take 104 days for the highest residue measured in liver (3.22 μg/g) to decline to just below detectable concentrations (≤ 0.02 μg/g). This is a conservative measure that does not take account of the overall elimination rate from the liver, including the more rapid initial phase. Consequently, to define a withholding period for feral pigs that are taken for human consumption in areas where diphacinone is present, allowance should be made for the possibility of higher diphacinone residues occurring in feral pigs than were measured in this trial. Therefore, a conservative withholding period of 156 days is recommended, based on the addition of half again to the estimated number of days for liver residues to become undetectable. Currently, the New Zealand Environmental Risk Management Authority (ERMA) recommends minimum caution periods for maintaining signage around areas to warn people that toxic baits have been used for pest control. If the baits contain coumatetralyl or diphacinone, the minimum caution period is 2 months after the baits have been retrieved from the place, or if the baits are not removed, 8 months after the last application of baits (ERMA 2004). DOC also specifies an 8-month minimum caution period for any of its pest control operations where diphacinone baits are not removed, or 4 months if diphacinone baits are removed (DOC 2004). While the maximum residue level (MRL) for brodifacoum in meat for human consumption has been set at 0.001 ppm (the current analytical limit of detection) in New Zealand (Clear 2003), no MRL has been set for diphacinone. Where game is to be taken from areas where vertebrate pesticides have been used, the New Zealand Food Safety Authority advises withholding periods of 3 years for brodifacoum and other second-generation anticoagulants, and 2 months for pindone, warfarin and other first-generation anticoagulants.

5. Conclusions

While no comparable half-life estimates for other anticoagulants in pigs are available, the results here are consistent with the findings of Fisher et al. (2003) for laboratory rats, in that diphacinone half-life in the liver of rats and pigs appears to be in the order of 3–5 days. Even though muscle and fat from feral pigs are the tissues most likely to be eaten by humans, it is most conservative to base risk assessments on the longest persistence of residues measured—the terminal half-life estimate for diphacinone in pig liver. On this basis, it is suggested that there is a risk of human exposure to diphacinone through the consumption of meat from feral pigs that have been exposed to diphacinone within a c. 160-day period. However, it is obviously important to field-check the period and extent of the risk estimated here, by monitoring feral pig tissues for residual diphacinone concentration over this period in areas where diphacinone baits have been used.

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